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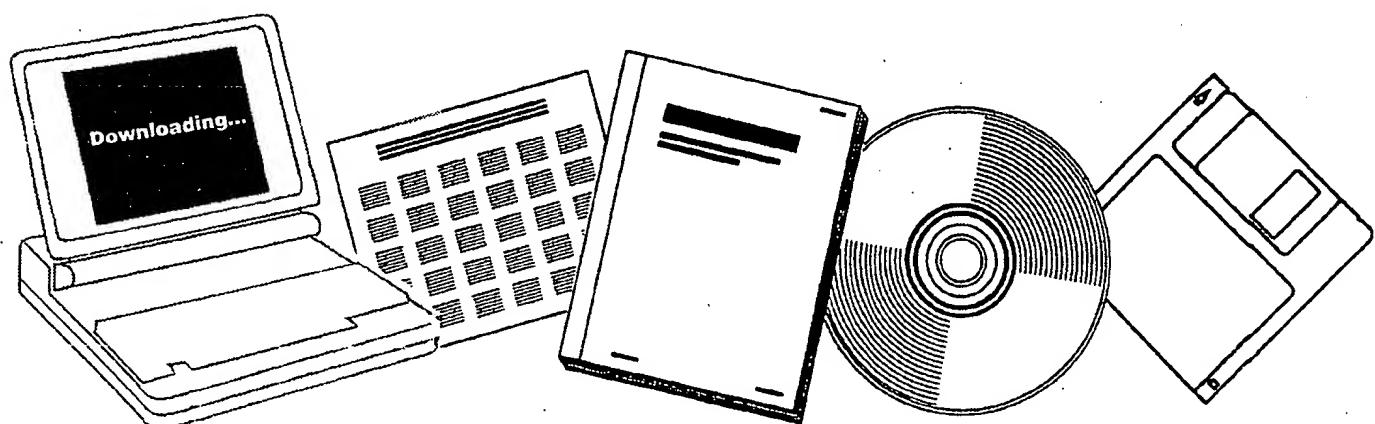
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TERT-BUTYLDIMETHYLSILYLOXY-SUBSTITUTED
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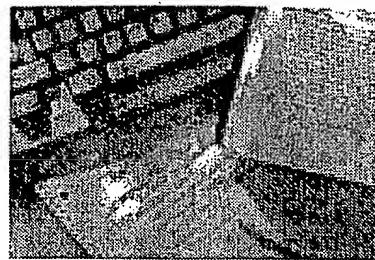
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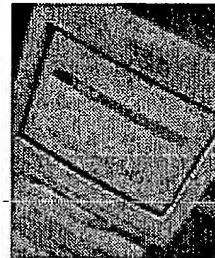
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TECHNICAL REPORT #3

**CHEMICAL AND ENZYMATIC TRIGGERING OF 1,2-DIOXETANES 2:
FLUORIDE-INDUCED CHEMILUMINESCENCE FROM
TERT-BUTYLDIMETHYLSILYLOXY-SUBSTITUTED DIOXETANES**

by

A. Paul Schaap, Tsae-Shyan Chen, Richard Handley, Renuka DeSilva, and B. P. Giri

Prepared for Publication

in

Tetrahedron Letters

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March 17, 1987

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CHEMICAL AND ENZYMATIC TRIGGERING OF 1,2-DIOXETANES. 2:
FLUORIDE-INDUCED CHEMILUMINESCENCE FROM
TERT-BUTYLDIMETHYLSILOXY-SUBSTITUTED DIOXETANES

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Abstract: Thermally stable 1,2-dioxetanes bearing *tert*-butyldimethylsilyloxyaryl groups have been prepared. Reaction of these dioxetanes with fluoride ion at ambient temperature in MeCN and DMSO generates chemiluminescence with efficiencies up to 25%.

In 1982 we demonstrated that chemiluminescence from a 1,2-dioxetane bearing a phenolic substituent could be triggered by the addition of base.¹ Deprotonation generates an unstable phenoxide-substituted dioxetane which decomposes 4.4×10^6 times faster than the protonated form. We have now used this initial observation to develop other methods for triggering the chemiluminescent decomposition of thermally stable dioxetanes. For example, we have recently shown that a naphthyl acetate-substituted dioxetane can be *enzymatically* cleaved in aqueous buffers using aryl esterase.² We now provide the first example of chemical triggering of silyloxy-substituted dioxetanes by fluoride in DMSO or MeCN to generate chemiluminescence with efficiencies up to 25%.

Dioxetanes **2a-c** were prepared by photooxygenation of the corresponding alkenes³ in CH_2Cl_2 using polymer-bound Rose Bengal⁴ (SENSITOX I) and a 1000-W high-pressure sodium lamp. After 15 - 30 min irradiation the sensitizer was removed by filtration and the solvent evaporated under vacuum. Recrystallization of the material from pentane or chromatography over silica gave the dioxetanes.⁵ Rate constants for the thermal decomposition of **2a-c** were obtained at 80 to 120 °C from measurements of the decay of chemiluminescence intensity of 10^{-4} M solutions in *o*-xylene.⁶ Chemiluminescence spectra from **2a-c** all exhibited a maximum at 437 nm indicating that the luminescence is derived from singlet excited adamantanone and not the phenyl or naphthyl esters. Rates showed variations of less than 3% and gave excellent Arrhenius plots ($r > 0.99$) with activation energies for **2a-c** of 29.7, 27.0, and 28.4 (± 1) kcal/mol, respectively (Table 1). Half-lives for **2a-c** at 25 °C are calculated to be several years.⁷ These results demonstrate the high degree of stabilization that can be obtained with sterically hindered adamantly-substituted dioxetanes.⁸

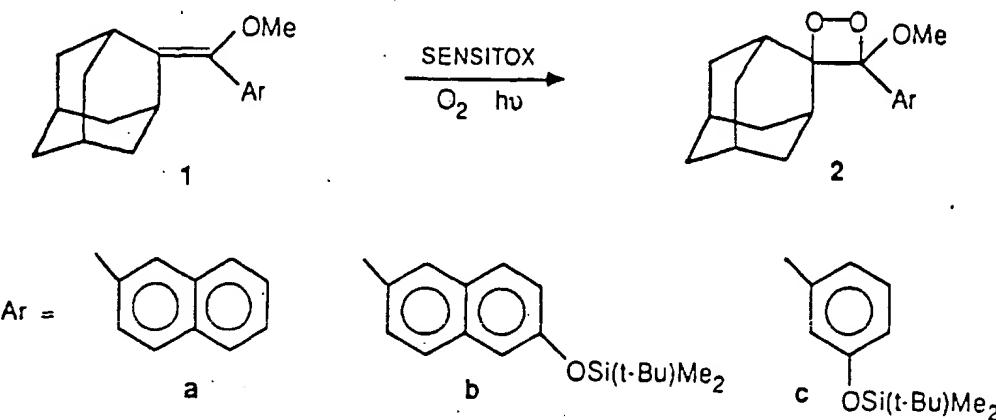


Table 1. Activation Parameters and Rates of Decomposition for 1,2-Dioxetanes 2a-c in o-Xylene.

dioxetane	E_a (kcal/mol)	log A	$k(\text{sec}^{-1})$ at 25 °C	half-life at 25 °C ^a
2a	29.7	13.2	3.17×10^{-9}	6.9 years
2b	27.0	11.7	8.72×10^{-9}	2.5 years
2c	28.4	12.6	5.74×10^{-9}	3.8 years

(a) Calculated from the Arrhenius plots.

Deprotection of silyl ethers with fluoride is a widely used reaction in modern organic synthesis.⁹ We have now used this procedure to generate the unstable, chemiluminescent aryloxide dioxetanes 3b and 3c from the thermally stable forms 2b and 2c, respectively. In a typical experiment injecting an aliquot of an o-xylene solution of dioxetane 2b into 3 mL of 0.001 M *n*-Bu₄NF in MeCN to give a final dioxetane concentration of 10⁻⁵ M produced a flash of blue chemiluminescence which decayed by a pseudo-first-order process with a half-life of less than 1 sec at room temperature. The spectrum of the resulting chemiluminescence exhibited a maximum at 470 nm which was identical to the fluorescence of 4b¹⁰ and the fluorescence of the spent dioxetane solution under these conditions. No chemiluminescence derived from adamantanone fluorescence appears to be produced. Analysis of the crude product mixture resulting from treatment of a sample of 2b with 1 equivalent of *n*-Bu₄NF by ¹H NMR and UV revealed only the expected cleavage products: adamantanone and the tetra-*n*-butylammonium salt of methyl 6-hydroxy-2-naphthoate in a 1:1 ratio.

The chemiluminescence quantum yield for the fluoride-triggered decomposition of dioxetane 2b was measured relative to the luminol standard¹¹ using a photon-counting apparatus.¹² Fluoride-triggered decomposition of 10⁻⁵ M solutions of 2b in MeCN at 25 °C produced chemiluminescence with a quantum yield of 4×10^{-5} which was independent of fluoride concentration in the range 10⁻² to 10⁻⁴ M. Correction for the fluorescence quantum yield of 4b in MeCN under identical conditions leads to a calculated chemiexcitation quantum yield of 1.1×10^{-4} or an efficiency for the formation of singlet excited 4b of 0.01% (Table 2).¹³

In contrast to the low chemiluminescence quantum yield on triggering the decomposition of 2b, we find that treatment of 2c with fluoride produces chemiluminescence with dramatically higher efficiency. Addition of excess *n*-Bu₄NF to 10⁻⁷ M solutions of dioxetane 2c in MeCN resulted in a rapid decomposition of 2c accompanied by bright blue chemiluminescence with a half-life of 5 sec at 25 °C. The pseudo-first-order rate constant was independent of fluoride concentration in the range 6.7×10^{-5} to 3.3×10^{-3} M. The spectrum of the resulting chemiluminescence exhibited a maximum at 470 nm in MeCN which matched exactly the fluorescence of 4c under these conditions. Similar experiments were conducted in DMSO (Figure 1).¹⁴ Quantum yields for the chemiluminescence of 2c with

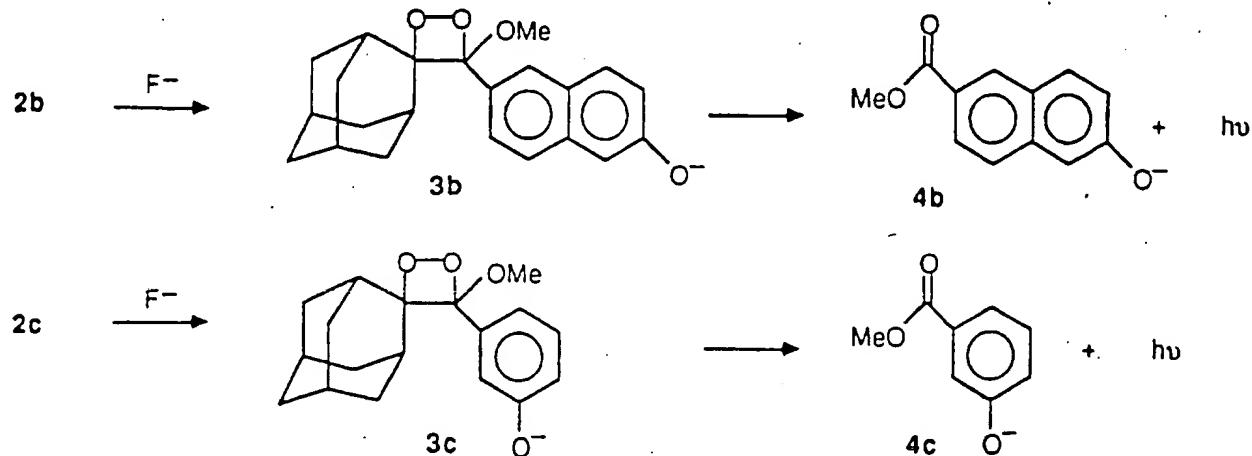


Table 2. Fluoride-Induced Chemiluminescence from Dioxetanes 2b and 2c.

dioxetane	solvent	Φ_{CL}^a	Φ_F^b	Φ_{CE}^c
2b	MeCN	4×10^{-5}	0.37	1.1×10^{-4}
2c	MeCN	0.094	0.21	0.45
2c	DMSO	0.25	0.44	0.57

(a). Chemiluminescence quantum yields. (b). Fluorescence quantum yields for cleavage products 4b and 4c relative to quinine sulfate with a value of 0.54.
 (c). Quantum yields for the formation of the singlet excited state of 4b and 4c.

fluoride were determined in MeCN and DMSO¹⁵ relative to the luminol standard and found to be 0.094 and 0.25, respectively (Table 2). Correction for the fluorescence quantum yields of 4c in these solvents gives efficiencies for the formation of singlet excited 4c of 45 and 57%, one of the highest singlet chemiexcitation efficiencies yet reported for a dioxetane.¹⁶

These results are readily explained by a mechanism initiated by cleavage of the Si-O bond by fluoride to generate the unstable dioxetanes 3b and 3c. The lack of any dependence of the rate of decay of the chemiluminescence on fluoride concentration suggests that the rate-limiting step under these conditions may be the cleavage of the aryloxide dioxetanes 3b and 3c.¹⁷ The rapid decomposition of these intermediates is induced by an intramolecular electron transfer from the strongly electron-donating phenoxide type substituent to the peroxide σ^* orbital.¹ Similar mechanisms have been proposed for the efficient chemiluminescence from dioxetanes bearing easily oxidized substituents.¹⁸ Chemiluminescence has also been observed from intermolecular electron-transfer reactions between peroxides and fluorescent hydrocarbons.¹⁹ The reason for the significant difference in the chemiexcitation efficiencies of 3b and 3c is currently under investigation. We have prepared the corresponding *p*-*tert*-butyldimethylsilyloxyphenyl-substituted dioxetane and found the chemiexcitation efficiency to also be less than 0.01% in that case.

We are continuing our work with dioxetane 2c and related derivatives with a view towards the possible use of this system as a convenient liquid light standard. Solutions of 2c prepared in *o*-xylene exhibit high stability and can be stored for long periods. A stock solution of 2c kept at room temperature gave identical results after one month. Typically, the calibration of a luminometer can be carried out by injecting 30 μ L of a 10⁻⁵ M solution of 2c into 3 mL of a 0.003 M solution of *n*-Bu₄NF in dry DMSO. The luminescence is emitted over a 20 sec period with 1-2% reproducibility for the total light emission.

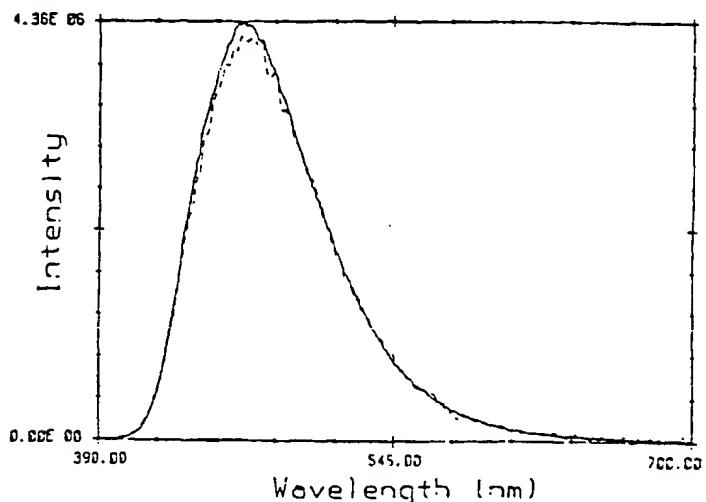


Figure 1. Chemiluminescence spectrum from fluoride triggering of dioxetane 2c in DMSO (---). Fluorescence spectrum of 4c under the same conditions (—).

Acknowledgments. Support of this research by the Office of Naval Research is gratefully acknowledged. We also wish to thank Mr. Marc Budrick for his assistance with the synthesis of the alkenes.

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2. Schaap, A. P.; Handley, R. S.; Giri, B. P. *J. Am. Chem. Soc.*, submitted for publication.
3. The preparation of the alkenes will be described in a subsequent paper.
4. Schaap, A. P., Thayer, A. L., Blossey, E. C., Neckers, D. C. *J. Am. Chem. Soc.* 1975, 97, 3741.
5. 4-Methoxy-4-(2-naphthyl)spiro[1,2-dioxetane-3,2'-adamantane] (2a): mp 116 °C (dec); ^1H NMR δ 0.9-2.0 (m, 12H), 2.22 (s, 1H), 3.11 (s, 1H), 3.24 (s, 3H), 7.0-8.3 (m, 7H); ^{13}C NMR δ 25.94, 26.07, 31.60, 31.72, 32.31, 33.08, 33.23, 34.88, 36.42, 50.00, 95.60, 112.33, 125.21, 126.47, 127.02, 127.63, 127.91, 128.67, 129.41, 132.13, 132.85, 133.61. 4-(6-*tert*-Butyldimethylsilyloxy-2-naphthyl)-4-methoxyspiro[1,2-dioxetane-3,2'-adamantane] (2b): mp 107 °C (dec); ^1H NMR δ 0.27 (s, 6H), 1.03 (s, 9H), 1.4-2.0 (m, 12H), 2.2 (s, 1H), 3.1 (s, 1H), 3.23 (s, 3H), 7.1-7.85 (m, 6H); ^{13}C NMR δ -4.33, 18.23, 25.67, 25.93, 26.06, 31.59, 31.69, 32.31, 33.04, 33.19, 34.86, 36.42, 49.94, 95.59, 112.44, 114.63, 122.58, 126.64, 128.50, 129.85, 130.11, 134.93, 154.59. 4-(3-*tert*-Butyldimethylsilyloxyphenyl)-4-methoxyspiro[1,2-dioxetane-3,2'-adamantane] (2c): oil, ^1H NMR δ 0.20 (s, 6H), 0.99 (s, 9H), 1.26-1.90 (m, 13H), 3.02 (s, 1H), 3.23 (s, 3H), 6.86-7.30 (m, 4H); ^{13}C NMR δ -4.34, 18.33, 25.77, 26.18, 26.07, 31.62, 31.70, 32.50, 33.00, 33.26, 34.80, 36.56, 49.94, 95.30, 111.91, 119.32, 121.26, 123.90, 129.18, 136.10, 155.86.
6. NMR experiments have shown that 2b-c undergo thermal decomposition to give 2-adamantanone and the expected silyloxyaryl ester.
7. Actual half-lives could be shorter if the solvents used for storage of the dioxetanes contained impurities which could lead to catalytic decomposition. Experiments are in progress to provide a direct measure of the stability of these dioxetanes at ambient temperature.
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9. Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* 1972, 94, 6190. For additional references to the deprotection of alcohols with fluoride, see: Greene, T. W. in "Protective Groups in Organic Synthesis," Wiley, New York, 1981.
10. Solutions of 4b and 4c were produced by treating the silyloxy-protected esters with fluoride.
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12. Quantum yields were determined using a luminometer constructed in our laboratory with an RCA A-31034A gallium-arsenide PMT cooled to -78 °C and Ortec photon-counting electronics.
13. For a discussion of the calculation of chemiluminescence quantum yields, see: Wilson, T. *Int. Rev. Sci.: Phys. Chem., Ser. Two* 1976, 9, 265.
14. Chemiluminescence spectra were acquired with a Spex Fluorolog spectrofluorometer. Correction for the decay of the chemiluminescence intensity during the wavelength scan was made by accumulating the spectrum in a ratio mode using an auxiliary detector to measure the total signal as a function of time.
15. Spectral grade MeCN was obtained from Burdick and Jackson Laboratories, Inc. Reagent grade DMSO from several sources gave identical quantum yields. We have used the trihydrate of *n*-Bu₄NF for these experiments. Other sources of fluoride produced similar results. We have noted that direct addition of fluoride to "old" samples of these solvents produces a very weak chemiluminescence in the absence of the dioxetanes.
16. For a listing of chemiexcitation efficiencies, see: Adam, W. in "Chemical and Biological Generation of Excited States," Eds. Adam, W.; Cilento, G., Academic Press, New York, 1982, Chapter 4.
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